# *Acanthamoeba pearcei* Nerad et al.

**50435™**

## Description

**Strain designation:** 205-1
**Deposited As:** *Acanthamoeba pearcei* Nerad et al.
**Type strain:** Yes

## Storage Conditions

**Product format:** Dried

## Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

## BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

## Growth Conditions

**Medium:**
ATCC Medium 997: Fresh water ameba medium
ATCC Medium 711: PYB
**Instructions for complete medium:**

**Media:** ATCC medium 997 optionally inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048ä).

**Alternate media:** ATCC medium 711 optionally inoculated with bacteria as above. (Note that ATCC medium 711 is a richer formula than ATCC medium 997 and will produce denser and faster bacterial growth. Excess bacterial growth can inhibit growth of amoebae.)

**Temperature:** 25°C
**Culture system:** Xenic

## Handling Procedures

### Establishing Cultures from Dried State

This strain comes dried on shredded filter paper. Dried samples can remain at room temperature for up to one week. If the culture will not be rehydrated within that period, store at 5°C until processed.

1. To rehydrate an ampule, aseptically add 1.0 ml of sterile distilled water to the inner shell vial. Aseptically remove the filter paper pellet with a pair of sterilized forceps and place it in the center of a plate of ATCC medium 997.
2. Using a Pasteur pipette, aseptically transfer the remainder of the liquid from the vial to the plate. Tease apart the filter paper pellet, and distribute evenly over the surface of the plate using a spread bar.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C. Trophozoites should be seen within 5-7 d.

**Culture maintenance:**

1. Streak an ATCC medium 711 plate with *Enterobacter aerogenes* (ATCC® 13048) and incubate at 35°C overnight.
2. Remove an agar block (~5 $mm^2$), with trophozoites or cysts, from the edge of an agar plate culture and invert the block at the edge of the freshly bacterized plate.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.
4. Repeat steps 1-3 at 10-14 d intervals.

Note: a monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

**Reagents for cryopreservation:**

Cryoprotective Solution
DMSO, 1.5 ml
Dryl's solution (or similar), 8.5 ml

**Cryopreservation:**

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest cells from a culture which is at or near peak density by adding 5 ml ATCC medium 5080 (Dryl's solution) and washing cells into suspension. Rub the

surface of the plate with a spread bar to detach adhering trophozoites.
3. Adjust the concentration of cells to at least 2 x $10^6$/ml in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
5. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)
7. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, aseptically transfer the contents of the ampule to the center of a fresh plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
9. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.

Follow the protocol for maintenance of culture.

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Acanthamoeba pearcei* Nerad et al. (ATCC 50435)

## References

References and other information relating to this material are available at www.atcc.org.

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## Revision

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